PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:	WO 92/10175
A61K 31/195, 9/14	A1	(43) International Publication Date:	25 June 1992 (25.06.92)

(21) International Application Number: PCT/EP91/02352

(22) International Filing Date: 9 December 1991 (09.12.91)

(30) Priority data:
22342 A/90 11 December 1990 (11.12.90) IT

(71) Applicant *(for all designated States except US):* DR. A. TORRE FARMACEUTICI S.R.L. [IT/IT]; Viale E. Forlanini, 15, I-20134 Milano (IT).

(72) Inventor; and
(75) Inventor/Applicant *(for US only) :* TORRE, Alberto [IT/IT]; Viale E. Forlanini, 15, I-20134 Milano (IT).

(74) Agent: MINOJA, Fabrizio; Studio Consulenza Brevettuale, Via Rossini, 8, I-20122 Milano (IT).

(81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CI (OAPI patent), CM (OAPI patent), CS, DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GN (OAPI patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC (European patent), MG, ML (OAPI patent), MN, MR (OAPI patent), MW, NL (European patent), NO, PL, RO, SD, SE (European patent), SN (OAPI patent), SU+,TD (OAPI patent), TG (OAPI patent), US.

Published
With international search report.

(54) Title: LYOPHILIZED AMINO ACID COMPOSITIONS CONTAINING GLUTAMINE

(57) Abstract

Lyophilized amino acid compositions containing glutamine, their preparation comprising solubilization, filtration and lyophilization and their use for parenteral feeding.

+ See back of page

+ DESIGNATIONS OF "SU"

Any designation of "SU" has effect in the Russian Federation. It is not yet known whether any such designation has effect in other States of the former Soviet Union.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	ES	Spain	MG	Madagascar
AU	Australia	FI	Finland	ML	Mali
BB	Barbados	FR	France	MN	Mongolia
BE	Belgium	GA	Gabon	MR	Mauritania
BF	Burkina Faso	GB	United Kingdom	MW	Malawi
BG	Bulgaria	GN	Guinea	NL	Netherlands
BJ	Benin	GR	Greece	NO	Norway
BR	Brazil	HU	Hungary	PL	Poland
CA	Canada	IT	Italy	RO	Romania
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU+	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		

LYOPHILIZED AMINO ACID COMPOSITIONS CONTAINING GLUTAMINE.

The present invention refers to amino acids compositions for the parenteral administration containing glutamine, in the form of sterile powder to be dissolved in sterile injectable solutions before use and to a method for the preparation thereof comprising solubilization, filtration and lyophilization.

Glutamine is a non-essential amino acid, since it may be synthesized in the body from other amino acids and precursors in an amount adequate to the physiological requirement.

It is the most abundant amino acid in the organism since it constitutes about 50% of the free intracellular amino acid pool of the muscular tissue.

The glutamine concentration, however, decreases rapidly when sepsis, trauma or other serious diseases occur, since the requirement is not sufficiently supported by the synthesis capacity of the organism. This decrease in glutamine levels causes a reduction of the protein synthesis, a decrease in the immune defenses and the atrophy of the intestinal mucosa.

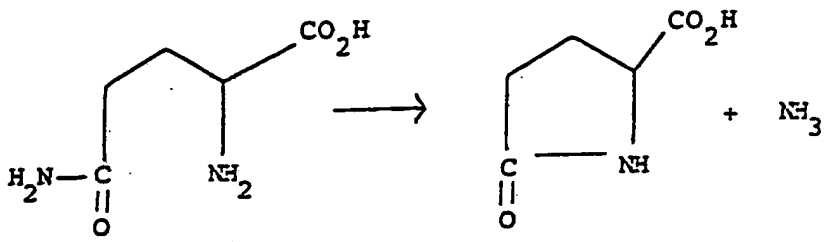
Meister (Physiol. Rev. 36:103-127, 1956) has already suggested the dietetic supplementation with glutamine to satisfy the increased metabolism of some cells or tissues.

Kapadia et al. (JPEN 6:583-589, 1985) reported that the glutamine infusion in a slightly catabolic model was able to preserve the intracellular levels of this amino acid.

Albers et al. (Akt. Ernährungsmed 9:147-149, 1984) were successful in sustaining the glutamine level in intra- and extra-cellular pools of muscular tissue by infusion of the dipeptide alanylglutamine in rats subjected to total parenteral feeding.

US 4,875,555 discloses the use of glutamine in the parenteral nutrition to prevent the catabolism of the muscular tissue, the atrophy of intestinal villi and of other dysfunctions. Even though many proposals for the preparation of glutamine formulations, including lyophilization of the amino acid, are generically included in the specification of US 4,875,555 no actual, practically applicable embodiment is given. In fact, in the used pharmacological tests, glutamine has always been administered in form of aqueous solution sterilized through a 0.22 µm membrane and mixed immediately before the use with other solutions for parenteral nutrition. The glutamine solution was preserved for 24 hours in the refrigerator so that the problem of the glutamine stability in the formulations for parenteral nutrition was not even addressed.

In fact, at present, infusion solutions of amino acids containing also glutamine are not available on the market, since this amino acid is very little soluble and is unstable in aqueous solution and it undergoes cyclization forming NH_3 and pyroglutamic acid.



This reaction occurs slowly at room temperature, but it is dramatically accelerated at sterilization temperature in autoclave. In order to overcome this problem, the use has been proposed, of stable derivatives in aqueous solution such as acetylglutamine and dipeptides, for instance alanylglutamine and glycylglutamine, which can be added to the infusional solutions containing the other amino acids.

But this solution has some drawbacks: acetylglutamine has a low bioavailability, whereas the dipeptides supply, besides glutamine, equimolar amounts of other amino acids which can be undesirable, such as glycine and which, anyhow, in view of the high dosage used, make the overall amino acidic pattern of the mixture unbalanced.

As another solution to this problem, the use of sterile lyophilized glutamine to be rehydrated or to be mixed with other powder components immediately before use was proposed. This solution is not of industrial application for the following reasons:

1) glutamine solubility is about 2.5%, therefore the lyophilization process is very expensive;
2) lyophilized glutamine is a very soft powder with very low density and poor flowability, therefore the use of automatic packaging machines is impaired;
3) lyophilized glutamine can be rehydrated only at a concentration lower than 2%, determining a water contribution non compatible with normal parenteral nutrition schemes; moreover, even so diluted solutions have a microparticle content which is

pharmaceutically unacceptable.

The present invention concerns the preparation of different injectable mixtures of amino acids and glutamine in lyophilized form to be dissolved in water before use or in the usual infusional solutions such as 5%, 10%, 30%, 50% glucose solutions. The invention provides the remarkable advantage of preventing the phenomenon of degradation of glutamine, thus allowing to preserve the amino acid compositions for a long time and making glutamine bioavailable immediately before use.

The pharmaceutical composition of the invention enables the preparation of formulations having a high glutamine content (up to 70%) in the presence of a generally balanced amino acidic content, avoiding the use of excipients, preservatives and stabilizers.

The compositions containing glutamine from 20 to 50% by weight are preferred. The compositions of the invention have a density $\geq$ 0.3 g/ml, a solubility of 10% in water and at least of 5% in glucose solutions with glucose concentrations ranging from 5 to 50%. The reconstituted solutions have a particulate count lower than these limits:

1.000	particles	$\geq$ 2 µ
100	"	$\geq$ 5 µ
50	"	$\geq$ 10 µ
4	"	$\geq$ 20 µ

The preparation process consists in the solubilization in water of the various amino acids present in the formulation and of glutamine, at a concentration from 10 to 20% w/v.

The solution is then filtered through sterilizing membrane, having pore size of 0.22 µm, at room temperature, and then it is freeze-dryed: the lyophilized product is distributed in the previously sterilized primary containers. All the operations are carried out in strictly aseptic conditions.

The amino acids contained in the solution act as glutamine lyophilization support and the final product, due to its density, flowability and physical characteristics, is suitable for industrial packaging. The rehydratation of the lyophilized product is immediate and complete. Injectable water or 5 to 50% glucose solution can be used as solvent. In spite of the amino acid content, ranging from 10% to 5%, microparticle count in the solution is far below the pharmaceutically acceptable limits.

According to the present invention, the lyophilized product allows to obtain solutions with a glutamine concentration higher than the one obtained with lyophilized glutamine only.

The compositions of the invention may be used in human therapy also in combination with glucides, lipids, vitamins, mineral salts according to the parenteral nutrition schemes. The percentages of amino acids and glutamine will be dependent on the patient's needs and on the physician's decision.

The invention is illustrated further by the following formulations exemplified in the table reporting the amino acid composition as percent by weight. It is evident that other amino acids can also be used according to specific nutritional needs.

TABLE 1

	I	II	III	IV
Isoleucine	8,5	8,5	6,2	6,2
Leucine	13,0	13,0	8,6	8,6
Lysine	7,0	7,0	7,9	7,9
(Lysine acetate)	(9,9)	(9,9)	(11,2)	(11,2)
Methionine	3,0	3,0	4,0	4,0
Phenylalanine	3,0	3,0	4,9	4,9
Treonine	5,0	5,0	5,0	5,0
Tryptophan	1,0	1,0	1,5	1,5
Valine	9,0	9,0	7,2	7,2
Total Ess. Am.	49,5	49,5	45,3	45,3
Arginine	2,5	1,0	2,1	
Hystidine	2,5	1,5	2,4	1,0
Alanine	7,5	6,5	4,0	1,5
Glycine	4,5	2,5	2,2	
Proline	8,5	6,0	2,0	1,2
Serine	5,0	3,0	2,0	1,0
Glutamine	20,0	30,0	40,0	50,0

Three batches corresponding to the formulation n. III were industrially produced.

The lyophilized product was distributed under nitrogen in doses of 25 g in 500 ml infusion bottles.

The solution was prepared by transferring aseptically 250 ml of water for injectable preparations or 500 ml of 5%, 10%, 20%, 30%, 50% glucose solutions in the lyophilized bottle.

The solution was sterile and apyrogenic with microparticle count within the limits stated by the Official Pharmacopoeia.

The stability of the compositions of the invention was

evaluated according to the following tests:
the amino acids assay by means of Amino acid Analyzer;
the glutamine titer by means of Amino acid Analyzer;
the pyroglutamic acid titer by HPLC.

The product remained unchanged at room temperature for more than 12 months.

The reconstituted solutions were stable for more than 15 hours at room temperature and for more than 48 hours at 4°C.

CLAIMS

1. Amino acids compositions for the parenteral administration containing glutamine in form of sterile powder to be dissolved in sterile injectable solutions before use.

2. Compositions according to claim 1, containing glutamine in amounts up to 70%.

3. Compositions according to claim 1, containing glutamine in amounts ranging from 20 to 50%.

4. Compositions according to any one of claims 1-3 having a density $\geq$ 0.3 g/ml, a solubility of 10% in water and at least of 5% in glucose solutions with glucose concentrations ranging from 5 to 50%, stability longer than 12 months at room temperature in the dry state and longer than 15 hours at room temperature or longer than 48 hours at 4°C, in reconstituted solution, said solution having a microparticle count lower than these limits:

1.000	particles	$\geq$ 2 µ
100	"	$\geq$ 5 µ
50	"	$\geq$ 10 µ
4	"	$\geq$ 20 µ.

5. A process for the preparation of lyophilized compositions of amino acids of claims 1-3 comprising:

a) solubilization of the amino acids and glutamine in water for injectable preparations;

b) filtration of the obtained solution through sterile membrane at room temperature;

c) freeze-drying of the filtrate;

d) distribution in sterile containers under nitrogen.

INTERNATIONAL SEARCH REPORT

International Application No PCT/EP 91/02352

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 31/195 A 61 K 9/14

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	WO,A,8701589 (BRIGHAM AND WOMEN'S HOSPITAL) 26 March 1987, see claims 1,7,9; page 11, lines 8-18; page 12, line 29 - page 13, line 15; page 57, line 26 - page 58, line 3 (cited in the application)	1,2,5

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07-02-1992	16. 03. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	Maria Peiz Maria Peiz

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. EP 9102352 SA 53877

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 11/03/92
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 8701589	26-03-87	AU-B- 599335	19-07-90
		AU-A- 6337886	07-04-87
		EP-A- 0238553	30-09-87
		JP-T- 63501214	12-05-88
		US-A- 5039704	13-08-91
		US-A- 4857555	15-08-89